Standard Operating Procedure for Primary Productivity Using ^{14}C:

Laboratory Procedures

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1.0 Scope and Application

This method is intended to determine primary productivity from Great Lakes waters.

2.0 Summary of Method

The radioactivity of the filter containing the algal cells is determined by liquid scintillation counting. Calculation of the productivity parameter requires information about the total inorganic carbon available in the incubation vessel, the length of time of incubation, the chlorophyll content of the incubated sample and specific activity of the radiotracer.

3.0 Safety and Waste Handling

3.1 ^{14}C is classified as a low-level beta emitter. Wearing personal protective laboratory gear at all times when in contact with the inoculated vials, can effectively prevent any exposure.

3.2 All spills of radioactive or suspected radioactive materials must be immediately reported to the CRL Safety and Health Officer and decontaminated immediately.

3.3 All radioactive samples and standards should be properly labeled with the isotope and activity indicated and properly stored in designated locations.

3.4 Under the Atomic Energy Act of 1954 a license is required designating the radioactive source, it's use as applicable to the laboratories and conditions by which the licensed material should be used. The current license (#12-10243-01) expires on March 31, 1995.

4.0 Apparatus

4.1 Packard TRI-CARB 4430 Liquid Scintillation Counter

4.2 Acetone

4.3 Kimwipes

4.4 Nucleopore filters 0.2 µm pore size, 2.5 cm diameter

4.5 Rubber bulbs (2)

4.6 9" Pasteur disposable pipets (sterilized)

4.7 200 mL volumetric flask
4.8 250 mL Flask with sidearm

4.9 Gelco 250 cc filtering system

4.10 Plastic filter plate

4.11 9 mm diameter rubber tubing

4.12 Glass funnel

4.13 Filter forcepts

4.14 1 mL volumetric pipet

4.15 Adjustable 10 mL Macropipettor

4.16 Gas-tight 20 mL vials

5.0 Reagents

5.1 Buffered water: Using Super Q water available in the chemistry lab, add 0.18 N NaOH dropwise until pH increases to 9.5. Prepare at least 400 mL of buffered water for every ampoule of ^{14}C one expected to use. Store buffered water in refrigerator at 5°C until ready to use in order to be at the same temperature as the ^{14}C solution.

5.2 Filtered water: (This procedure may be performed in the field)

5.2.1 After ensuring that all glassware has been properly cleaned, wrap in brown paper and autoclave on high temperature setting (wrap cycle). Keep glassware in wrapping paper until directly before use to avoid contamination.

5.2.2 Set up filtration apparatus using Nucleopore 0.2 µm filters and cover filter funnel until ready to use.

5.2.3 Pour cooled buffered water (Section 5.1) into filtration funnel and turn on vacuum pressure. Cover the funnel while the solution is being filtered.

5.2.4 Replace the filter as necessary and prepare at least 400 mL of filtered water per ampoule ^{14}C.

5.2.5 Following the filtration, disassemble the filtration apparatus and cover the filter flask with parafilm until ready to use.

5.2.6 Decant approximately 20 mL of the filtered water into a second beaker to serve as wash water. Cover the beaker with parafilm.

5.3 0.5 N Hydrochloric Acid Solution: Add 4.25 mL of concentrated hydorchloric acid to 800 mL of deionized water in a 1 L volumetric flask. Adjust volume to one liter with deionized water.

6.0 Stock Solution

6.1 Ensure all the liquid in the top portion of the ^{14}C ampoule has been shaken down into the lower Section. Using a diamond pencil or a rough-sided file, score the weak edge of the ^{14}C ampoule.

6.2 Carefully, placing the ampoule between thumb and forefinger, break off the top at the weakened neck. Pipette the contents into a 200 mL volumetric flask using the sterilized Pasteur pipettes.

6.3 Using a disposable glass pipet, carefully rinse out both the top and bottom portions of the ampoule using the filtered water and add wash solution to the flask. Repeat three times.

6.4 Adjust volume in the flask to 200 mL using filtered water. With stopper firmly in place, vortex for several seconds to mix solution.

6.5 Pour the stock solution into a cleaned and autoclaved Nalgene container with an opening wide enough to accommodate a macropipettor.

6.6 Dispense 13 mL of the stock solution into a 20 mL gas tight vial and screw cap on tightly. Fifteen vials can be prepared from each ampoule which is diluted. For each set of incubated bottles, one vial will be used for inoculation.

6.7 Label each vial with "Stock #". Assign a chronological number beginning with number one, indicating the first ampoule diluted for that year. Store in a radioactively labeled (specific activity) container at 5°C until ready for analysis.

6.8 For quality control measures, using a volumetric pipet, dilute a 1 mL sample of the freshly made stock solution into 300 mL water (pH 8.5).

6.9 Vortex dilution several times and dispense a 1 mL sample into a scintillation vial.

6.10 Add 20 mL of Ecoscint plus 1 mL phenoethylamine. Clearly label cap with year and the stock number which the sample represents. This number will correspond with each stock solution which is subsequently made. This will be analyzed along with the other samples to determine the beginning activity of the stock solution.

6.11 If possible, set aside one vial from each stock solution to determine the final activity, repeating Steps 6.5 through 6.7.

7.0 Instrument Calibration Procedure

7.1 The Liquid Scintillation counter should be calibrated once per month and directly prior to sample analysis. The results of the monthly calibration are included in the biology report.

7.2 Refer to the Standard Operating Procedures for calibration instruction.

8.0 Analytical Procedures

8.1 After 24 hours, remove scintillation vials and clean the outside of each vial by holding the vial by the cap and wiping the outside walls with an acetone-impregnated tissue and drying with a clean dry tissue. Due to the high flammability of acetone, this procedure should be performed underneath a hood.

8.2 Remove the scintillation vial from sample storage and place them in the scintillation trays and allow the samples to dark adapt for 24 hours.

8.3 Ensure all that the numbers on the caps are readable and in chronological order.

8.4 Place the vials, handling only the caps, into the counter.

8.5 Each series of samples, field standards and background (laboratory blanks) should be counted for 20 minutes. Check output screen on scintillation counter for the value of two sigmas.

8.6 Counting efficiency should be obtained to obtain results as DPM. Most scintillation counters output results as DPM if a set of quenched standards are provided.

9.0 Sample Calculations

9.1 The carbon uptake can be calculated as follows:

$$C^{12} = \frac{C^{14}U \times C^{12}A \times 1.06}{C^{14}A \times t}$$

Where:

t	=	*exposure time (hours)*
C^{12}	=	*carbon uptake rate (mg/C/L/Hr)*
$C^{14}U$	=	*sample activity (DPM)*
$C^{14}A$	=	*added activity (DPM)*
$C^{12}A$	=	*inorganic carbon available (mg/L) determined by means of pH and alkalinity or by direct determination of total inorganic carbon*
1.06	=	*isotope effect constant*

9.2 Normalize the carbon uptake rate to chlorophyll content:

$$\frac{C^{12}\ uptake}{CHl}$$

Where: CHl = *Chlorophyll concentration mg/l*

9.3 For each sample incubated, report

9.3.1 Unadjusted production rate, mg C/L/hr.

9.3.2 Normalize production rate, mg C/L/hr/mg chlorophyll

9.3.3 Light intensity at which sample was incubated

9.3.4 Length of incubation

10.0 Waste Calculations

10.1 While in the field, it is possible to make a rough estimate of the activity for each waste container (See *Standard Operating Procedure for Primary Productivity Using ^{14}C Field Procedure* Section 2.2.2). This estimate is necessary for shipping and storage purposes and can be approximated in µCi/mL.

10.2 A ^{14}C waste form must be properly filled out for each survey. A example of this form can be found in the appendix. The actual waste values are required to be reported in total µCi and the form submitted to the CRL Health and Safety Officer.

10.3 Following analysis at CRL in the Scintillation Counter, the DPM values for the waste are attainable, thus allowing the actual µCi values to be determined.

10.4 For each cubie, multiply the DPM value by the waste volume, V, to obtain the total DPM per cubie.

10.5 Average the DPM values for all Total Activity vials. This factor corresponds with 0.01667 µCi, the activity of 1 mL (5 µCi) of the stock solution added to a 300 mL sample. The Total Activity is the actual specific activity of a 1 mL sample from the incubation bottles.

10.6 Multiply V by 0.01667 µCi and divide this by the Total Activity average. Repeat for each waste cubie.

10.7 Waste calculations should show individual µCi values as well as the total amount of waste generated per survey. See attached Appendix 1 for sample calculations.

11.0 Quality Control

Prior to the sample analysis in the Liquid Scintillation Counter, the background and efficiency of the counter is calculated. See *Standard Operating Procedures for the Liquid Scintillation Counter*.

Appendix 1.

Radioactivity calculations for ^{14}C waste water (approximate)

1 mL stock solution = 5 µCi
1 mL added to 300 mL HO
5 µCi/300mL = **0.016671µCi/mL**

#1 (46547.4 DPM/mL)(18000mL) = 8.38×10^8 DPM
#2 (41184.7 DPM/mL)(18000mL) = 7.41×10^8 DPM
#3 (45052.6 DPM/mL)(14400mL) = 6.48×10^8 DPM
#4 (51063.5 DPM/mL)(14400mL) = 7.35×10^8 DPM
#5 (4534.88 DPM/mL)(7200mL) = 3.26×10^7 DPM
#6 (13642.3 DPM/mL)(18000mL) = 2.45×10^8 DPM
#7 (14388.6 DPM/mL)(16000mL) = 2.30×10^8 DPM

0.01667µCi corresponds to **36117.92 DPM** (avg. of all vials)

#1 (8.38×10^8 DPM)(O.O1667µCi/mL)/36117.92 = 386.70 µCi
#2 (7.41×10^8 DPM)(O.O1667µCi/mL)/36117.92 = 342.15 µCi
#3 (6.48×10^8 DPM)(O.O1667µCi/mL)/36117.92 = 299.43 µCi
#4 (7.35×10^8 DPM)(O.O1667µCi/mL)/36117.92 = 339.38 µCi
#5 (3.26×10^7 DPM)(O.O1667µCi/mL)/36117.92 = 15.07 µCi
#6 (2.45×10^8 DPM)(O.O1667µCi/mL)/36117.92 = 113.34 µCi
#7 (2.30×10^8 DPM)(O.O1667µCi/mL)/36117.92 = 106.26 µCi
Total: 1602.33 µCi

1.60 mCi -Total waste